RAD [redacted] ENGINEERING REPORT (10/01/18)

INITIAL REVIEW ENGINEERING REPORT [redacted]

TERA: R18-0001 ENGINEER: Macek/JAS (AFD Review)

SUBMITTER: Arizona Center for Algae Technology and Innovation (AzCATI) at Arizona State University (ASU)
AzCATI Testbed facility
7418 Innovation Way South
Mesa, Arizona 85212

MICROORGANISMS:

Recipient/host (p. 9):

i. *C. Sorokiniana* DOC1412

Donor (p. 9):

i. SNRK2_PACE_plasmid vector introduced to improve photosynthetic efficiency and biomass in the recipient organism (p. 9).

GEM: The TERA is referred to by PACE_Cs1412_SNRK2 (p. 7).

PV (CFU/yr): 7.2×10^{15} CFU/yr

Basis: The submission indicates that the miniponds will be run semi-continuously between 800-1,000 L at a density ranging from 0.1 to 1.0 g/L. The ponds will be inoculated between 0.1 to 0.2 g/L and biomass will be harvested from the ponds when the density reaches between 0.4 to 1 g/L. The submitter expects to harvest 50-80% of biomass in the ponds between 1 to 10 times during the experiment at a maximal density of 1.0 g/L. Each harvest may take place between 5 and 15 days after inoculation. The submitter expects to utilize at 6 ponds (p. 56). As a conservative estimate, RAD assumes 6 ponds and 10 harvests per pond. The technical contact indicated a harvest density of 0.2 to 0.5 g/L (~5 to 12 x 10^7 CFU/mL)(same as [redacted]).

Based on this information, RAD calculates the PV to be:

Total PV = (6 ponds/harvest)(10 harvests/yr)*(1,000 L/minipond)(1,000 mL/L)*(12 x 10^7 CFU/mL)

Total PV = 7.2 x 10^{15} CFU/yr

Per the submission, the experimental trial will last approximately 60 days (p. 62). Therefore, any releases/exposures for the above-calculated PV will occur over 60 days/yr, and will not occur year-round.

USE: The subject microorganism will be used in this experiment for two purposes: 1) to investigate the translatability of phenotypes from lab to field and 2) to understand how microalgae migrate and affect natural plankton communities (p. 57).

SUMMARY:

The specific insertions / deletions (and corresponding effects) are discussed in detail on pages 8-56 of the submission.

The submission states that the TERA will be used in this experiment for two purposes: 1) to investigate the translatability of phenotypes from lab to field and 2) to understand how microalgae migrate and affect natural plankton communities (p. 57).

The trial will take place at Arizona Center for Algae Technology and Innovation (AzCATI) at Arizona State University (ASU) AzCATI Testbed facility in Mesa, Arizona. The TERA will be cultivated in outdoor miniponds and sent for analysis/testing at the ASU lab or freeze-dried bulk harvest will be sent to other users within the PACE consortium.

When the cell density in the minipond reaches the desired level, the TERA will be harvested and centrifuged. The concentrated algae paste will be bottled before being frozen and transported to the lab (within secondary

containment) for continued storage until analyses are performed.

NOTES AND KEY ASSUMPTIONS

The 1997 Generic Scenario for Biotechnology Premanufacture Notices was referenced in this IRER. In addition RAD referenced the May 12, 2000 technical policy memorandum on "Efficiency of Autoclaves for Laboratory-Scale Equipment" which assumes that for cases involving steam sterilization of laboratory scale equipment (<10 liters) in an autoclave, RAD will regard the potential for release of live microorganisms as negligible.

Per September 16, 2013 "Updating CEB'S Method for Screening-Level Estimate of Dermal Exposure," RAD updated the single-hand surface area from 420 cm^2 to 535 cm^2.

The submitter previously submitted [redacted], for the same recipient microorganism (*C. sorokiniana*) and same use. The technical contact was called, who indicated that responses are the same as for the previous case [redacted] (see contact report).

The submitter indicates that to ensure that the subject microorganism is completely removed from the test site after the experiment has been completed – all equipment (miniponds, trap ponds, and used sample containers) will be washed overnight in a 5% bleach solution, which has been shown to be sufficient to inactivate *C. sorokiniana.* (p. 67). However, because no quantitative inactivation data were available, RAD conservatively assumed 99% inactivation as a low-end estimate, based on values reported in Appendix A of the GS – Inactivation of Bacteria with Chlorine.

RAD assesses a 100% release scenario. The TERA will be treated with bleach and/or autoclaved before disposal into the sewer system (p. 59).

Sporulation

The technical contact indicated that to their knowledge, the TERA does not form spores; therefore, spore releases were not assessed.

INITIAL REVIEW ENGINEERING REPORT [redacted]

TERA: R18-0001

PROCESSING: Propagation and Harvesting of TERAs in Miniponds

Sites/Locations: 1

AzCATI Testbed facility
7418 Innovation Way South
Mesa, Arizona 85212

Days/yr: 60

Basis: The submission estimates that the experiment will occur over 60 calendar days (p. 62), which is consistent with the estimated maximum exposure duration of 60 days/yr (p. 59).

The submission estimates that the ponds will be harvested between 1 to 10 times during the experiment and each harvest may take place between 5 and 15 days after initial inoculation (p. 57).

PROCESS DESCRIPTION

The subject microorganism is received at the AzCATI laboratory under the PACE Material Transfer Agreement, and are subject to an NIH R&D exemption (40 CFR parts 725.234 and 725.235). The strain will be transported on agar plates and scaled up in closed systems. (p. 58).

Laboratory Propagation

The recipient and subject microorganisms will enter the AzCATI laboratory on agar plates and will be scaled up in through small shaker flasks, 800mL bubble columns, to 2 ft by 2 ft flat panel photobioreactors with a 2.0 inch light path operated at maximum volume of 10L. When the appropriate density (2.5 g/L) is reached in the 10L flat panel photobioreactors, the submitter will combine culture in approved containers with secondary containment to transport seed culture from laboratory to field site (approximately 100 meters away) to be used to inoculate the ponds (p. 58).

Pond Inoculation and Propagation

The ponds will be inoculated to a starting density of at least 0.1g/L at a depth of 800-1,000L, (entry weight of 0.24-0.3kg biomass per pond)(p. 58). Pond surface area is 4.2 m^2 (p. 56)

Samples will be inactivated by treatment with 5% bleach solution and autoclaved. Bulk cultures will also be inactivated by treatment with bleach and disposed into the sanitary sewer (per large scale culture disposal protocol) (p. 59).

The miniponds will be cultivated, samples will be taken, and biomass will be harvested when the culture reaches a density of 0.4g/L to 1.0 g/L ash free dry weight (AFDW) (p. 56).

Workers at the field site will dose nitrogen and phosphorus as needed based on daily nutrient analysis. Nitrogen and phosphorus will be dosed at 25% to 100% of original media levels (provided in Table 8) after pond harvest or to maintain media levels (p. 69).

Sampling

Methods of cultivation will consist of performing sampling on the miniponds, trap ponds and other AzCATI fieldsite ponds as outlined in Table 9 of the submission. The primary monitoring and grab sampling of the ponds daily/weekly will involve up to 2 fulltime AzCATI staff with part time student worker support to monitor pH and temperature in the ponds, check on in-situ YSI sensors (for water quality monitoring), and acquiring the grab samples for further processing in the laboratory. Most samples will be processed on the same day, but some may take up to 5 days. Nutrients will need to be periodically added, in particular after pond harvest events and will be reset to target levels per the growth response of the ponds (p. 69).

All field test samples will be inactivated with 5% bleach or autoclaving before disposal via sewage system and samples for future analyses are freeze-dried, which also renders the cells non-viable (p. 67)

Samples that are transported from laboratory and field site are labeled with designated information including, batch ID, source location, date, strain ID, collectors name and purpose of the sample. Samples that are transported between laboratory and field site will be placed into secondary containment (p. 66-67)

Harvesting
Ponds will be harvested by the field site as needed based upon dry weight data, as communicated by the AzCATI. Harvest will be triggered by biomass density 0.4 g/L to 1.0 g/L AFDW and the harvested volume will be calculated to target a new dry weight of 0.4 g/L (once the harvested volume is replaced by new media) (p. 56).

The material harvested will be dewatered to a slurry (via centrifugation) and the concentrated algae paste will be freeze dried or stored as frozen (p.57).

Supernatant from centrifugation will also be treated at 5% bleach level and discarded down the sanitary sewer (p. 69).

All cultures are harvested using a Beckman-Coulter Avanti J Centrifuge series and JLA 8.1000 fixed-angel rotor 1L bottle assembly (or similar). After centrifugation, the supernatant is poured out manually without removing the pellet. The biomass pellet is scraped from the 1L centrifuge bottle using scoop or scraping tool. (SOPs Attachment – pg. 10-12).

Once the field experiment has been terminated, all biomass will be inactivated by bleaching or autoclaving. All equipment will be cleansed of the subject microorganism (including all sample containers, ponds, etc.) by bleaching, autoclaving and will be discarded as necessary. Any pond spill will be contained within the containment area, treated with bleach solution, and liquid disposed of in the sewage system. (p. 69)

ENVIRONMENTAL RELEASE SUMMARY

WATER:

1) From: Equipment cleaning

Amount:
1.4×10^{12} CFU/yr
1.4×10^{11} CFU/day over 10 days/yr

Basis: Equipment cleaning and will occur during harvesting (1 – 10 times during the experiment). Submission indicates that all equipment will be cleansed of the subject microorganism

(including all sample containers, ponds, etc.) by bleaching and will be discarded as necessary (p. 69). RAD uses the total PV and RAD 2% residual model to calculate these releases.

- 7.2×10^{15} CFU/yr (PV per TERA, see calcs above)
- Equipment cleaning and 10 harvests/yr and 1 release day/harvest
- 2% multiple vessel residual model
- Inactivation efficiency of 99%. The technical contact expects that the TERA will be fully inactivated, but submitter did not provide cell kill data. RAD conservatively assumes a 2-log kill, as a low end inactivation estimate, based on values reported in Appendix A of the GS – Inactivation of Bacteria with Chlorine.

WR = (PV)(1 - 0.99)(2% equipment residual)

= $(7.2 \times 10^{15}$ CFU/yr)(1 - 0.99)(0.02)

= 1.4×10^{12} CFU/yr

Per day:

= $(1.4 \times 10^{12}$ CFU/yr)/((10 harvest/yr)* (1 release day/harvest))

= 1.4×10^{11} CFU/day

2) From: Centrifuge supernatant waste

Amount:

7.2×10^{11} CFU/yr
7.2×10^{10} CFU/day over 10 days/yr

Basis: Centrifugation will occur during harvesting (1 – 10 times during the experiment). Submission indicates supernatant from the centrifugation of the harvested material will be dosed with 5% bleach before disposal (p. 69).

- 7.2×10^{15} CFU/yr (PV per TERA, see calcs above)
- Equipment cleaning and 10 harvests/yr and 1 release day/harvest
- The submission did not specify the centrifuge separation efficiency. RAD assumes 99% efficiency (consistent with past case [redacted] and [redacted]. This yields a 1% residual that would be released with the supernatant.
- Inactivation efficiency of 99%. The technical contact expects that the TERA will be fully inactivated, but submitter did not provide cell kill data. RAD conservatively assumes a 2-log kill, as a low end inactivation estimate, based on values reported in Appendix A of the GS – Inactivation of Bacteria with Chlorine.

WR = (PV)(1 - 0.99)(1% filtered supernatant)

= $(7.2 \times 10^{15}$ CFU/yr)(1 - 0.99)(0.01)

= 7.2×10^{11} CFU/yr

Per day:

= $(7.2 \times 10^{11}$ CFU/yr)/((10 harvest/yr)* (1 release day/harvest))

= 7.2×10^{10} CFU/day

3) From: Sample Wastes

Amount: negligible

Basis: The submission states that collected samples will be inactivated with a 5% bleach solution and autoclaved. (p. 67) RAD protocol (per May 2000 technical memo – see Key Notes and Assumptions) is to assume that small-scale autoclaving (steam sterilization

treatment) results in negligible CFU releases.

AIR:

1) From: Bioaerosol emissions

Amount:

7.2×10^6 CFU/yr
1.2×10^5 CFU/day, over 60 days/yr

Basis: Air releases can occur from aerosols generated from agitation due to sparge gas or paddlewheels. Currently, RAD does not have methodology for estimating these types of releases; therefore RAD estimates this potential release using the methodology described in the Biotech GS for fermentor exhaust gas.

- 10 batches/yr (see calcs above)
- 6 days/batch (60 days/yr divided by 10 batches/yr)
- 1,000,000 mL Minipond Broth Volume (1,000 L, per submission (p. 56))
- 6 ponds (per submission (p.56))
- Aerosolization factor (dimensionless factor indicating the proportion of CFU-containing aerosol particles in the size range of 1 to 10 microns formed per initial number of cells in the liquid volume considered) of 1×10^{-9} (RAD GS default)
- 12×10^7 CFU/mL (max final broth concentration per technical contact)
- 0% Removal Efficiency (no engineering controls are employed to reduce air emissions)

Calculations (based on std. RAD methodology for fermentor exhaust gas):

$AR_{FO,\ total} = ([CFU_B])(AF)(1-n_R)(V_B)$

$AR_{FO,\ total} = (12 \times 10^{7}\ CFU/mL)(1 \times 10^{-9})(1-(0))$
$(1{,}000{,}000\ mL/pond)(6\ ponds/batch)$

$AR_{FO,\ total} = 7.2 \times 10^{5}\ CFU/batch$

Annual total

$= (7.2 \times 10^{5}\ CFU/batch)(10\ batch/yr)$

$= 7.2 \times 10^{6}\ CFU/yr$

Per day:

$= (7.2 \times 10^{6}\ CFU/yr\ per\ TERA)/(60\ days/yr)$

$= 1.2 \times 10^{5}\ CFU/day\ per\ TERA$

2) From: Fugitive Emissions From Process Unit Equipment (e.g., centrifuge)

Amount:

7.2×10^{6} CFU/yr
1.2×10^{5} CFU/day, over 60 days/yr

Basis: Air releases can occur from aerosols generated from process equipment, such as centrifuges.

- 10 batches/yr (see calcs above)
- 6 days/batch (60 days/yr divided by 10 batches/yr)
- 1,000,000 mL Minipond Broth Volume (1,000 L, per submission (p. 56))
- 6 ponds (per submission (p.56))
- Aerosolization factor (dimensionless factor indicating the proportion of CFU-containing aerosol particles in the size range of 1 to 10 microns formed per initial number of cells in the liquid volume considered) of 1 X 10^{-9} (RAD GS default)

- 12×10^7 CFU/mL (max final broth concentration per technical contact)
- No inactivation before centrifugation ($n_I = 0$)

Calculations (based on std. RAD methodology for process unit operation equipment):

$AR_P = ([CFU_B])(V_B)(1-n_I)(AF)$

$AR_P = (12 \times 10^7 \text{ CFU/mL})(1 \times 10^{-9})(1-(0))$
$(1{,}000{,}000 \text{ mL/pond})(6 \text{ ponds/batch})$

$AR_P = 7.2 \times 10^5$ CFU/batch

Annual total

$= (7.2 \times 10^5 \text{ CFU/batch})(10 \text{ batch/yr})$

$= 7.2 \times 10^6$ CFU/yr

Per day:

$= (7.2 \times 10^6 \text{ CFU/yr})/(60 \text{ days/yr})$

$= 1.2 \times 10^5$ CFU/day

3) From: Fugitive Emissions During Sampling

Amount: Negligible

Basis: Per the 1997 Biotech GS, potential fugitive air releases from sampling have shown to be either undetectable or several orders of magnitude lower than those from fermentor off-gas, centrifugation, and filtration. Therefore, compared to other air emission sources, sampling air emissions are considered to be negligible.

INCINERATION: Not expected

Basis:

The submission indicates that all equipment, samples, and materials that contain the TERA will be treated with bleach and/or autoclaving, and disposed to the sewage system (p. 59). This is consistent with the 1997 Biotech GS, which does not specify any expected releases to incineration.

WATER:

4) From: Paste Disposal (from Bulk Harvest)

Amount:

7.0×10^{13} CFU/yr
7.0×10^{12} CFU/day over 10 days/yr

Basis: Technical contact indicated that all samples besides bulk harvest will be completely used. Technical contact indicated bulk harvest will be either centrifuged, freeze-dried, and shipped to other people in the consortium or bleached and sent to POTW. However, technical contact could not estimate amount sent to POTW. Submission states that bulk culture will be inactivated by treatment with bleach and disposed of into the site's sanitary sewer (p. 59).

- 7.2×10^{15} CFU/yr (PV per TERA, see calcs above)
- 97% of PV disposed (assuming 100% release scenario: 100% - 2% equipment cleaning – 1% centrifuge waste = 97%)(Note, aerosol releases are several orders of magnitude lower than equipment cleaning and separation wastes and were not considered here).
- 10 releases per yr (per frequency of bulk harvest over 60 days)
- Inactivation efficiency of 99%. The technical contact expects that the

TERA will be fully inactivated, but submitter did not provide cell kill data. RAD conservatively assumes a 2-log kill, as a low end inactivation estimate, based on values reported in Appendix A of the GS – Inactivation of Bacteria with Chlorine.

$WR = (PV)(1-0.99)(97\%)$

$= (7.2 \times 10^{15}\ CFU/yr)(1 - 0.99)(0.97)$

$= 7.0 \times 10^{13}\ CFU/yr$

$= (7.0 \times 10^{13}\ CFU/yr)/(10\ days)$

$= 1.2 \times 10^{13}\ CFU/day$

OCCUPATIONAL EXPOSURE

Number of Total Workers: up to 13

Basis: The submission provided worker estimates in the following table (p. 59):

Worker Activity	PPE	# of Workers Exposed	Maximum Duration (hr/day)	Maximum Duration (day/yr)
Scale-up and Inoculation	Gloves, lab glasses, lab coat, long pants, and closed-toe shoes.	2	2	30
Routine pond monitoring (pH, temperature)		2	2	60
Grab Samples (scope, OD, AFDW, qPCR, proximate analysis, etc.)		2	2	60
Sample processing		3	6	45
Pond harvests/resets		2	6	10
Terminating Experiment		2	4	3

Source: Page 59.

Note that all exposures were assessed for 13 workers, assuming that the same worker does not perform more than one activity. Therefore, the number of workers exposed may be less depending on whether the same workers perform multiple activities.

Days/yr: up to 60 (see above)

PPE: The submission indicates that proper personal protective equipment will be worn by all on-site

staff (as required by ASU EH&S regulations), which includes: gloves, lab glasses, lab coat, long pants, and closed-toe shoes (p. 68). SOP Attachments provided indicate that rubber (latex or nitrile) gloves should be used. No respiratory protection is specified- only to work in well-ventilated areas. The SOP attachments indicate use of face mask during use of muriatic acid during pond teardown.

INHALATION (bioaerosols):

1) From: Pond Harvesting (Centrifuge)

Amount of Exposure:

Up to 8 workers (initial application, pond monitoring, sampling, and pond harvesting in table).

360 to 2,500 CFU/day, 10 days/yr

Basis:

RAD's 1997 Biotech Generic Scenario includes area monitoring data collected by NIOSH in a fermentation facility. The GS recommends estimating potential inhalation exposures by taking the most applicable monitoring data and multiplying it by an estimate of the exposure duration. Per technical contact, bulk harvest centrifugation will occur on site.

- $[CFU]_{WA}$ = 47.6 CFU/m^3 (NIOSH average of geometric means at centrifuge) to 329 CFU/m^3 (NIOSH max at centrifuge, per GS – unknown whether indoor or outdoor)
- 10 days/yr (see above)
- I = 1.25 m^3/hr
- H = hours per day = 6 (Submission)
- 8 workers/site

Calculations (based on std. RAD methodology):

$E_I = (I)(h)([CFU]_{WA})$ (per GS)

= (1.25 m^3/hr)(6 hr/day)(47.6 to 329 CFU/m^3)

= 360 (avg) to 2,500 CFU/day (worst case)

2) From: Sample Processing and Experimental Termination

Amount: Negligible

Basis: Per the 1997 Biotech GS, potential fugitive air releases from sampling are shown to be either undetectable or several orders of magnitude lower than those from fermentor off-gas, centrifugation, and filtration.; Therefore, compared to other air emission sources, sampling air emissions are considered to be negligible and inhalation exposures negligible.

DERMAL:

1) From: Daily Sample Processing

Amount of Exposure:

4 workers/site (sample processing and experimental termination in table).

4.8 x 10^7 to 1.3 X 10^8 CFU/day, up to 60 days/yr

Basis:

The submission and technical contact indicated that sample processing will occur daily. Per the biotech GS, the potential dermal dose rate is the product of RAD standard dermal exposure assessment factors and the CFU concentration in the appropriate process stream.

- $[CFU]_B$ = 12 x 10^7 CFU/mL (broth concentration, per technical contact)
- C = liquid transfer - 1 hand, the RAD standard dermal factor is (535 cm^2/day)(0.7 to 2.1 mg/cm^2)(1 g/1000 mg)(1 mL/g) = 0.4 to

1.1 mL/day (RAD assumes 1 hand transfer for sample analysis – note revised hand surface area per 2013 guidance – see Key Notes and Assumptions)

- 60 days/yr (see above)
- 4 workers/site

Calculation:

$(12 \times 10^7$ CFU/mL)(0.4 to 1.1 mL/day)

$= 4.8 \times 10^7$ to 1.3 X 10^8 CFU/day

2) From: Loading of Algae paste into Bottles

Amount of Exposure:

2 workers/site (sample processing in table)

9.0×10^7 to 2.6 X 10^8 CFU/day, 10 days/yr

Basis:

The technical contact indicated that algae paste would be bottled during harvesting. Per the biotech GS, the potential dermal dose rate is the product of RAD standard dermal exposure assessment factors and the CFU concentration in the appropriate process stream.

- $[CFU]_P = 12 \times 10^7$ CFU/mL (broth concentration, per technical contact) (technical contact was unable to provide a paste concentration)
- C = liquid transfer - 2 hands, the RAD standard dermal factor is (1,070 cm^2/day)(0.7 to 2.1 mg/cm^2)(1 g/1000 mg)(1 mL/g) = 0.75 to 2.2 mL/day (Note revised hand surface area per 2013 guidance – see Key Notes and Assumptions)
- 10 days/yr (i.e., one day per harvest)
- 2 workers/site

Calculation:

$(12 \times 10^7 \text{ CFU/mL})(0.75 \text{ to } 2.2 \text{ mL/day})$

$= 9.0 \text{ X } 10^7 \text{ to } 2.6 \text{ X } 10^8 \text{ CFU/day}$

[redacted] **CONTACT REPORT** **(TERA: R18-0001)**

Submitter:	Arizona Center for Algae Technology and Innovation
DATE:	September 24, 2018
Person Contacted:	John McGowen
Affiliation:	Director of Operations and Program
Telephone:	480-727-1472
Caller:	Jason Sese
Affiliation:	ERG for RAD

Q: Are there any significant change between this TERA and the [redacted] Are responses to questions for [redacted] valid for this TERA?

A: No, there are no changes from the previous TERA – this is the same recipient organism so all procedures and targets are identical as [redacted].

Relevant Questions/Answers from [redacted] are included below

Q: Can you translate the biomass densities during harvest to colony forming units (CFU)/mL?

A: Starting density at 0.05 g/L max 0.6 g/L. The cell size is about 5 microns and the morphology is round. Mr. McGowen indicated that harvest concentrations would range from ~5 x 10^7 to 12 x 10^7 CFU/ml (0.2 to 5 g/L).

Q: When the samples (1-4 L) are harvested and centrifuged for transport to the lab, what is the concentration of the TERA (in CFU/mL) in the algae paste? Can you estimate the percent of PV used for paste for analyses, liquid monitoring, and unused paste? Can you estimate that number of analysis days per harvest?

A: Not including bulk harvest. Grab samples for analysis run volume is between 800L to 1000L and likely 3 subject organism ponds and 3 wild type (recipient) organism ponds. Any that is spun down goes for proximate analysis and all gets processed as freeze-dried material (4 L). Pull enough

to yield 0.5 g on dry weight basis and consume all with analysis.

The facility removes 75% of the pond for harvest for reset. Bulk harvest we will centrifuge it down on site, the containment area is big enough (outdoors) to centrifuge down. 75% of the 3 subject organism ponds yields about 5kg of paste (at 20% solids) and 1kg on a dry weight basis. Paste from bulk harvest is freeze-dried and ship to other people in the consortium. If they don't centrifuge, the bulk harvest is bleached and sent to POTW. Technical contact did not have an estimate for the percent of the bulk harvest sent to POTW.

Q: Does the TERA form spores?
A: No.

Q: On page 58, the submission states, "For the activities described herein, we will also utilize at least 6 and up to 12 trap ponds for the dispersion testing"; however, on page 56, the submission states that "We will utilize 6 ponds total." Can you clarify how many ponds are used? What is the total capacity of the miniponds and trap ponds (4.2 m^2 surface area; what is the depth)?
A: The facility will use 6 miniponds for the TERA. Depth target is 20 cm or 25cm, can accommodate a running depth up to 30 cm (40L per cm of depth). AzCATi would not run ponds any lower than 15 cm.